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CD4+ T cells specific for tumor antigens have been less well characterized than CD8+ T cells. There appears to be two main reasons for this discrepancy; 1) $CD8^+$ T cells isolated from tumor (tumor infiltrating lymphocytes; TIL) preferentially expand in the presence of anti-CD3 and IL-2, and 2) $CD4^+$ T cells isolated from a tumor environment appear to be defective in signaling and therefore may not have the capacity to proliferate to tumor/tumor-associated Ag. We will attempt bypass these limitations by using a novel approach to costimulate a tumor specific $CD4^+$ T cell memory response. Recently, we found that $CD4^+$ T cells isolated near the tumor sites of patients with melanoma and head and neck cancer expressed the OX-40 receptor, but not cells in the periphery of these same patients. It is our hypothesis that these $OX\text{-}40^+$ T cells were recently activated in vivo in response to tumor antigens. If a costimulatory signal could be provided to these $OX\text{-}40^+$ T cells by the OX-40 ligand, then clonal expansion of CD4+ T cells specific tumor should occur. In this proposal we will characterize OX-40 expression by human and mouse $CD4^+$ T cells specific for breast cancer and attempt to expand them both in vivo and in vitro with MHC II^+ tumors transfected with the OX-40 ligand, in essence making the tumor an antigen presenting cell capable of priming CD4 T cell immunity.

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FOREWORD

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Andrew Wiley 8/28/98

PI - Signature Date

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Introduction:

Approximately 45,000 women will die from breast cancer in the US in the upcoming year. The death of these women will be a direct result of the inadequacy of current adjuvant therapy for women with early stage breast cancer and of systemic therapy for women with metastatic breast cancer. Clearly, there needs to be an effective novel therapy to employ at the time when standard therapy with surgery, radiotherapy and chemotherapy has failed. A novel approach is the use of immunotherapy to boost a persons immune system to specific breast cancer antigens. The immune system is designed specifically for the deployment of effector cells throughout the body for the purpose of recognizing and destroying entities that appear to be foreign or harmful to self (e.g. breast cancer cells). Therefore learning how to enhance the immunogenicity of breast cancer in patients with tumors is the goal of the proposed research.

We have focused on $CD4^+$ T cell immunity in breast cancer to help boost self-immunity to tumors. We have found in an autoimmune model that the $CD4^+$ T cells invading the target organ specifically express a cell surface protein termed OX-40 (CD134). When the $OX\text{-}40^+$ T cells were sorted from the inflamed tissue they were found to be the autoantigen reactive cells. We and others have shown that engaging the OX-40 cell surface protein causes a costimulatory event that leads to T cell proliferation and expansion. Recently, we have found that $CD4^+$ T cells isolated near the tumor sites of patients with melanoma and head and neck cancer expressed the OX-40 protein, but the peripheral lymphocytes in these patients did not express OX-40.

The focus of the work performed in this grant proposal is to examine OX-40 expression in patients with breast cancer at both the site of inflammation and in the periphery. Once we have established that there are $OX\text{-}40^+$ cells in patients with breast cancer we intend to focus on OX-40 specific therapies in order to increase the amount of tumor reactive $CD4^+$ T cells. Ultimately, increased numbers of breast cancer specific T cells should enhance immunity to tumors leading to increased tumor-free survival in these patients. The summary of the first year's work is presented in the "body" of this annual review.

Body:

We have analyzed surgical samples from 6 patients with breast cancer for the expression of the OX-40 antigen expressed on $CD4^+$ T cells. In two of the patients we were able to obtain blood, lymph node and tumor to stain for OX-40 expression. In the 4 other patients we were just able to obtain blood and the tumor. One problem that we encountered is that the pathologists stage the breast cancer patients by the amount of lymph nodes that are positive for tumor, therefore it is hard to obtain lymph node samples for staining. The peripheral blood from all of the patients were negative for OX-40. In the two patients that we were able to obtain lymph nodes, the $CD4^+$ T cells were slightly positive for OX-40 expression <4%. The tumor samples were digested and the tumor infiltrating lymphocytes were dual stained for CD4 and OX-40. Two of six tumors samples were positive for OX-40 expressing CD4 T cells and in one of the samples 10% of the CD4 cells were $OX\text{-}40^+$, while the other positive sample showed approximately 5% of the CD4 cells were $OX\text{-}40^+$. The presence of the $OX\text{-}40^+$ cells within the surgical samples suggested that reagents designed to stimulate T cells through the OX-40 receptor would be beneficial in expanding tumor reactive T cells within breast cancer patients. To test this hypothesis we have utilized two different murine breast cancer models to assess the clinical efficacy of engaging the OX-40 receptor in vivo.

The two breast cancer models include the SM1 cell line which will form a solid tumor when injected s.c. and does not metastasize and the 4T1 tumor which forms a solid tumor when injected s.c. and does spontaneously metastasize to lung, liver, and lymph node. We have taken two basic approaches to engage the OX-40 receptor in vivo: 1) Injecting anti-OX-40 on days 3 and 7 after injecting the tumor s.c., and 2) Transfecting the tumor directly with the OX-40 ligand and injecting the tumor s.c. The transfection efficiency for the SM1 tumor was quite low and we were not able to obtain stable clones that expressed the OX-40 ligand. Therefore, we treated animals that had received SM1 tumor s.c. with anti-OX-40 on days 3 and 7 post-tumor inoculation (control animals received rat Ig). The animals were then scored for tumor-free survival for 90 days. All the control animals had to be sacrificed within 40 days of tumor inoculation, while 25% of the anti-OX-40 treated animals remained

tumor-free (N=35/group). The data for this observation was presented at the 1998 American Association of Cancer Research meeting in New Orleans (1) and is currently being written in journal format for a publication submission. The tumor-free animals were rechallenged with tumor and never developed any signs of disease, suggesting that the anti-OX-40 treated animals had developed tumor-specific memory. Currently, we have found a combination therapy with anti-OX-40 that works 3-fold better than anti-OX-40 alone. The combination therapy will be attempted in the SM1 system in the upcoming year.

The 4T1 tumor was successfully transfected with the OX-40 ligand and we obtained 3 clones that express high/medium/low levels. We injected the high and medium clones into animals s.c. and compared growth of the primary tumor to the parental 4T1 line. The primary tumors from both groups grew at identical rates but the transfected tumors were not metastatic (like the parental 4T1) when we looked for tumor in lung and lymph node. Currently, we are repeating this experiment with another set of transfectants (both OX-40 ligand$^{+/-}$) to make sure this is a valid observation and not just an artifact of 4T1 subcloning.

Conclusions:

The work accomplished has shown the expression of the OX-40 antigen on $CD4^+$ T cells invading human breast cancer and in T cells within tumor draining lymph nodes. The OX-40 expression was restricted to the inflammatory site because peripheral blood lymphocytes from these patients were OX-40 negative. This prompted us to perform murine studies with reagents designed to stimulate $OX\text{-}40^+$ cells in vivo. Our initial studies using anti-OX-40 in animals with tumor or transfecting tumors with the OX-40 ligand show promise for beneficial therapeutic effects. In the next year we will try to refine our technique in order to optimize the OX-40 specific therapies in the murine system. Ultimately, our goal will be to use OX-40 specific therapies to enhance tumor-specific immunity in women with breast cancer.

Final Report:

Bibliography:

1. Morris, A., Vetto, J., Funatake, C., and Weinberg, A. (1998) Breast cancer immunity in mice treated with the OX-40 ligand. American Association for Cancer Research Metting, New Orleans, LA, Abstract# 3615, page 193.

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